Binding Domain Hybrid		Activation Domain Hybrid: Sentrin
Fas		
	wt (191-319AA)	++
	△ 15 (191-304AA)	++
	△ 23 (191-296AA)	-
	(V238N)	-
TNFR1		
	wt (326-426AA)	++
	△ 14 (326-412AA)	++
	△ 20 (326-406AA)	-
CD40		
	(216-277AA)	-
FADD/MORT1		
	(1-208AA)	-

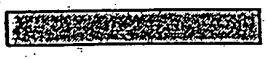
Activation Domain Hybrid		Binding Domain Hybrid: Fas (191-319AA)
Sentrin	(1-101AA)	++
	(1-70AA)	-
	(1-23AA)	-
	 (24-97AA)	-
Ubiquitin	(1-76AA)	-
Nedd8	(1-76AA)	-

FIG. 1A

7.5-
4.4-
2.4-
Heart
Brain
Placenta
Lung
Liver
Skeletal Muscle
Kidney
Pancreas
Spleen
Thymus
Prostate
Testis
Ovary
Small Intestine
Colon
Peripheral Blood
-Sentrin
-β-Actin

FIG. 1B

A

```
   1  CGAGGCGTAGCGGAAGTTACTGCAGCCGCGGTGTTGTGCTGTCGGGAAGGGGAAGGATTT
  61  GTAAACCCCGGAGCGAGGTTCTGCTTACCCGAGGCCGCTGCTGTGCGGAGACCCCCGGGT
 121  GAAGCCACCGTCATCATGTCTGACCAGGAGGCAAAACCTTCAACTGAGGACTTGGGGGAT
                      M  S  D  Q  E  A  K  P  S  T  E  D  L  G  D     15
 181  AAGAAGCAAGGTGAATATATTAAACTCAAAGTCATTGGACAGGATAGCAGTGAGATTCAC
      K  K  E  G  E  Y  I  K  L  K  V  I  G  Q  D  S  S  E  I  H     35
 241  TTCAAAGTGAAAATGACAACACATCTCAAGAAACTCAAAGAATCATACTGTCAAAGACAG
      F  K  V  K  M  T  T  H  L  K  K  L  K  E  S  Y  C  Q  R  Q     55
 301  GGTGTTCCAATGAATTCACTCAGGTTTCTCTTTGAGGGTCAGAGAATTGCTGATAATCAT
      G  V  P  M  N  S  L  R  F  L  F  E  G  Q  R  I  A  D  N  H     75
 361  ACTCCAAAAGAACTGGGAATGGAGGAAGAAGATGTGATTGAAGTTTATCAGGAACAAACG
      T  P  L  E  L  G  M  E  E  E  D  V  I  E  V  Y  Q  E  Q  T     95
 421  GGGGGTCATTCAACAGTTTAGATATTCTTTTTATTTTTTTCTTTTCCCTCAATCCTTTT
      G  G  H  S  T  V  *101
 481  TTATTTTTAAAAATAGTTCTTTTGTAATGTGGTGTTCAAAACGGAATTGAAAACTGGCAC
 541  CCCATCTCTTTGAAACATCTGGTAATTTGAATTCTAGTGCTCATTATTCATTATTGTTTG
 601  TTTTCATTGTGCTGATTTTTGGTGATCAAGCCTCAGTCCCCTTCATATTACCCTCTCCTT
 661  TTTAAAAATTACGTGTGCACAGAGAGGTCACCTTTTTCAGGACATTGCATTTTCAGGCTT
 721  GTGGTGATAAATAAGATCGACCAATGCAAGTGTTCATAATGACTTTCCAATTGGCCCTGA
 781  TGTTCTAGCATGTGATTACTTCACTCCTGGACTGTGACTTTCAGTGGGAGATGGAAGTTT
 841  TTCAGAGAACTGAACTGTGGAAAAATGACCTTTCCTTAACTTGAAGCTACTTTTAAAATT
 901  TTGAGGGTCTGGACCAAAAGAAGAGGAATATCAGGTTGAAGTCAAGATGACAGATAAGGT
 961  GAGAGTAATGACTAACTCCAAAGATGGCTTCACTGAAGAAAAGGCATTTTAAGATTTTTT
1021  AAAAATCTTGTCAGAAGATCCCAGAAAAGTTCTAATTTTCATTAGCAATTAATAAAGCTA
1081  TACATGCAGAAATGAATACAACAGAACACTGCTCTTTTTGATTTTATTTGTACTTTTTGG
1141  CCTGGGATATGGGTTTTAAATGGACATTGTCTGTACCAGCTTCATTAAAATAAACAATAT
1201  TTGTCAAAAATCGTACTAATGCTTATTTTATTTTAATTGTATAGAAAGAAAAAAATGCCT
1261  AAAATAAGGTTTTCTTGCATAAATACTGGAAATTGCACATGGTACAAAAAAAAAATGCCT
1321  AAATTACTGTACAGGGATGATGTTAATGACTTTGGAGCACTGAAAGTTACTGAAGTGCCT
1381  TCTGAATCAAGGATTTAATTAAGGCCACAATACCTTTTTAATACTCAGTGTTCTGTTTTT
1381  TTTAAAAACTTGATATTCCCGTATGGTGCATATTTGATACAGGTACCCAATCATGTTGGA
1441  TAAATGGGCATGCCAGCC
```

FIG. 2A

0837E0 n96n8n60

```
                                                                                                  60
                1
Sentrin         MSD    QEAKPST EDLGDKKEGE YIKLKVIGQD SSEIHFKVKM TTHLKKLKES YCQRQGVPMN
SMT3            MSDSEVNQEAKPEV KP-EVKPETH -INLKV-SDG SSEIFFKIKK TTPLRRLMEA FAKRQGKEMD
Ubiquitin                                  MQIFVKTLT GKTITLEVEP SDTIENVKAK IQDKEGIPPD
Nedd-8                                     MLIKVKTLT GKEIEIDIEP TDKVERIKER VEEKEGIPPQ
BAG-1                                                                ---VQDLAQL VEEATGVPLP

                                                                                             100
                                           61
                                           SLRFLFEGQR IADNHTPKEL GMEEEDVIEV YQEQTGGHST V
Sentrin                                    SLRFLYDGIR IQADQTPEDL DMEDNDIIEA HREQIGGATY
SMT3                                       QQRLIFAGKQ LEDGRTLSDY NIQKESTLHL VLRLRGG
Ubiquitin                                  QQRLIYSGKQ MNDEKTAADY KILGGSVLHL VLALRGG
Nedd-8                                     FQKLIFKGKS LKE-------
BAG-1
```

FIG. 2B

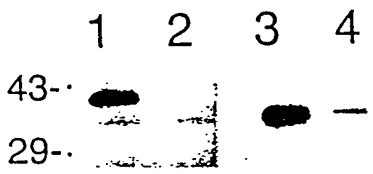


FIG. 3

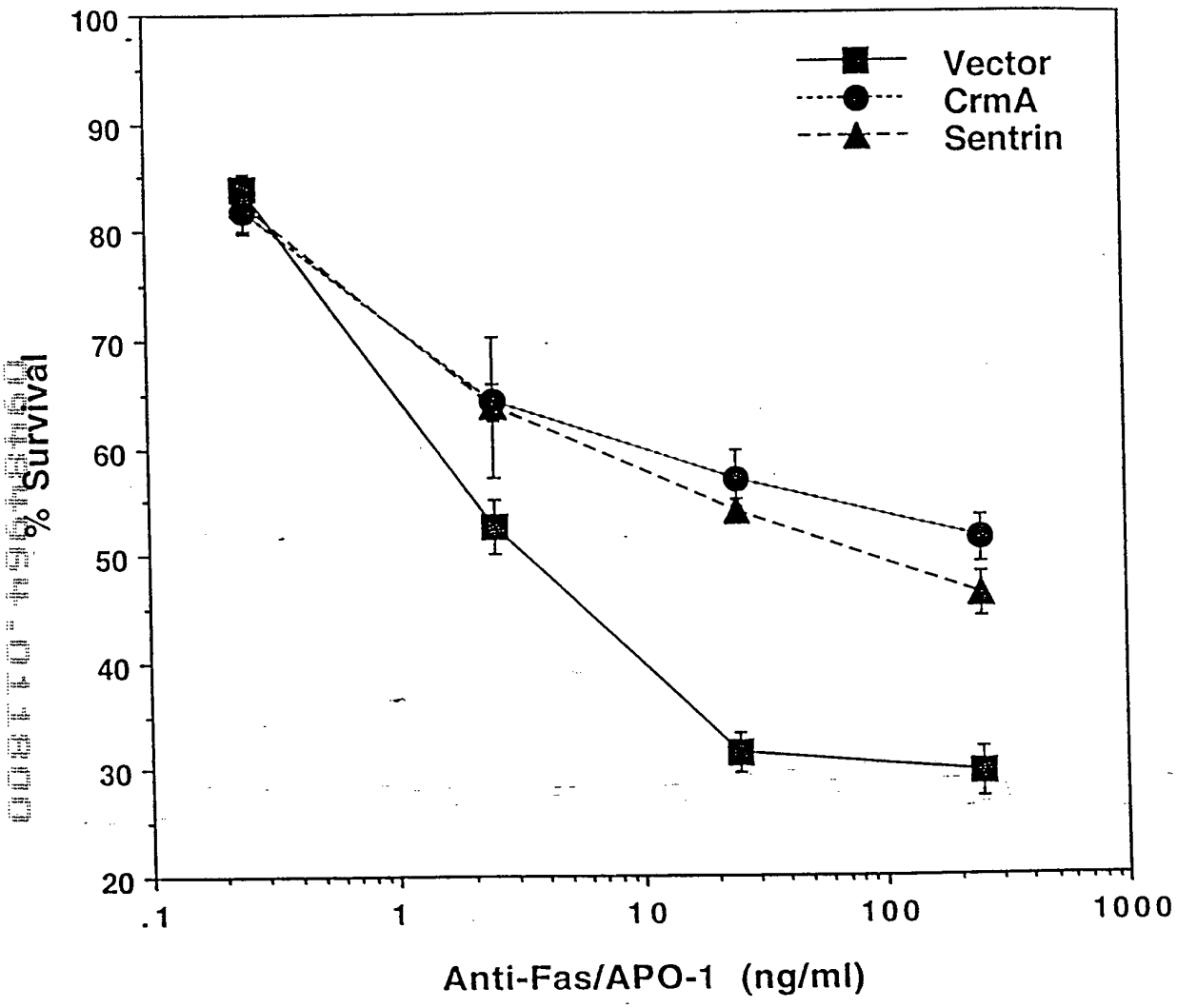

100
90
80
70
60
50
40
30
20
% Survival
.1
1
10
100
1000
Anti-Fas/APO-1 (ng/ml)
Vector
CrmA
Sentrin

FIG. 4A

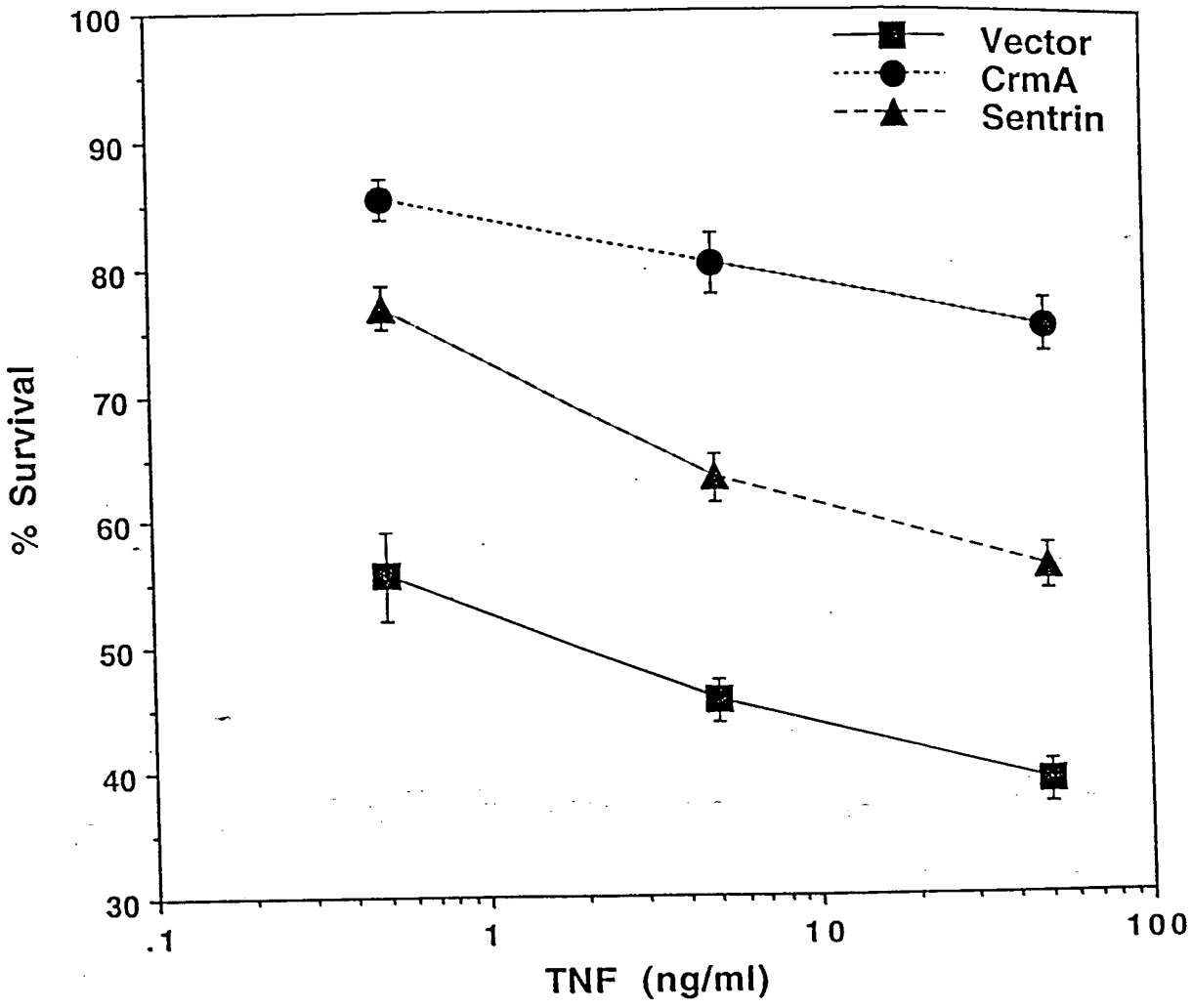

100
90
80
70
60
50
40
30
% Survival
.1
1
10
100
TNF (ng/ml)
Vector
CrmA
Sentrin

FIG. 4B

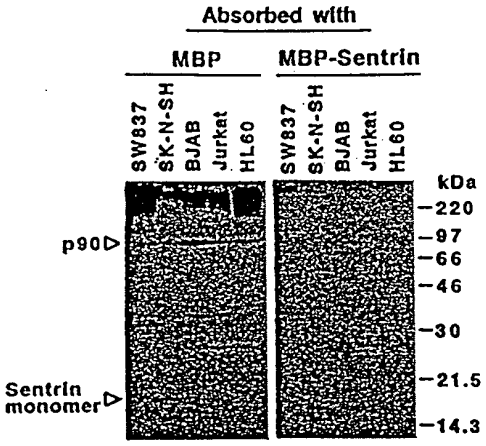

Absorbed with
MBP
MBP-Sentrin
SW837
SK-N-SH
BJAB
Jurkat
HL60
SW837
SK-N-SH
BJAB
Jurkat
HL60
kDa
220
97
66
46
30
21.5
14.3
p90
Sentrin monomer

FIG. 5

FIG. 6A

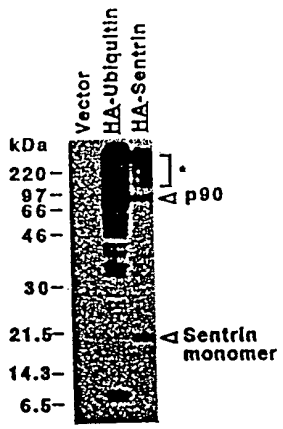

Vector
HA-Ubiquitin
HA-Sentrin
kDa
220
97
66
46
30
21.5
14.3
6.5
p90
Sentrin monomer

FIG. 6B

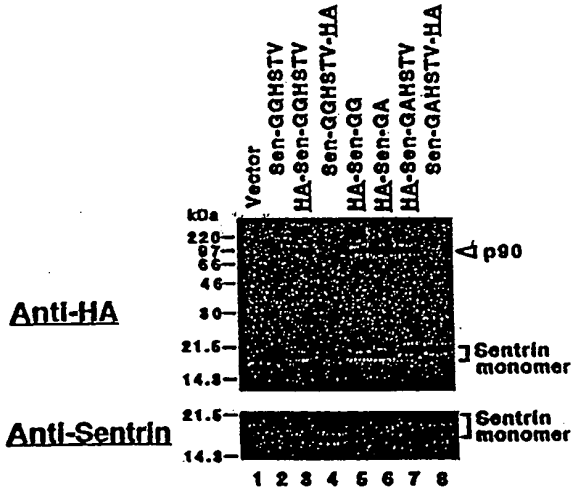

Vector
Sen-GQHSTV
HA-Sen-GQHSTV
Sen-GQHSTV-HA
HA-Sen-GQ
HA-Sen-GA
HA-Sen-GAHSTV
Sen-GAHSTV-HA
kDa
220
97
66
46
30
21.5
14.3
p90
Sentrin monomer
Anti-HA
Anti-Sentrin
1 2 3 4 5 6 7 8

FIG. 7A

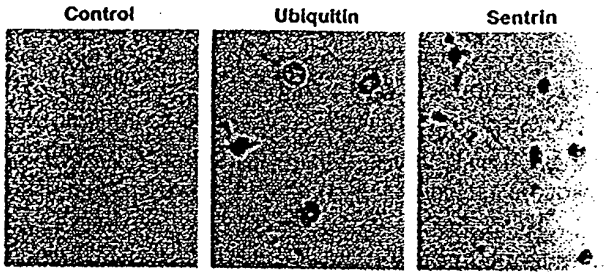
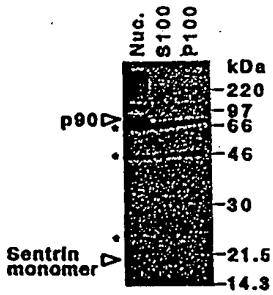

Control
Ubiquitin
Sentrin

FIG. 7B


Nuc.
S100
P100
kDa
220
97
66
46
30
21.5
14.3
p90
Sentrin monomer

Interaction of Sentrin with Ubc9

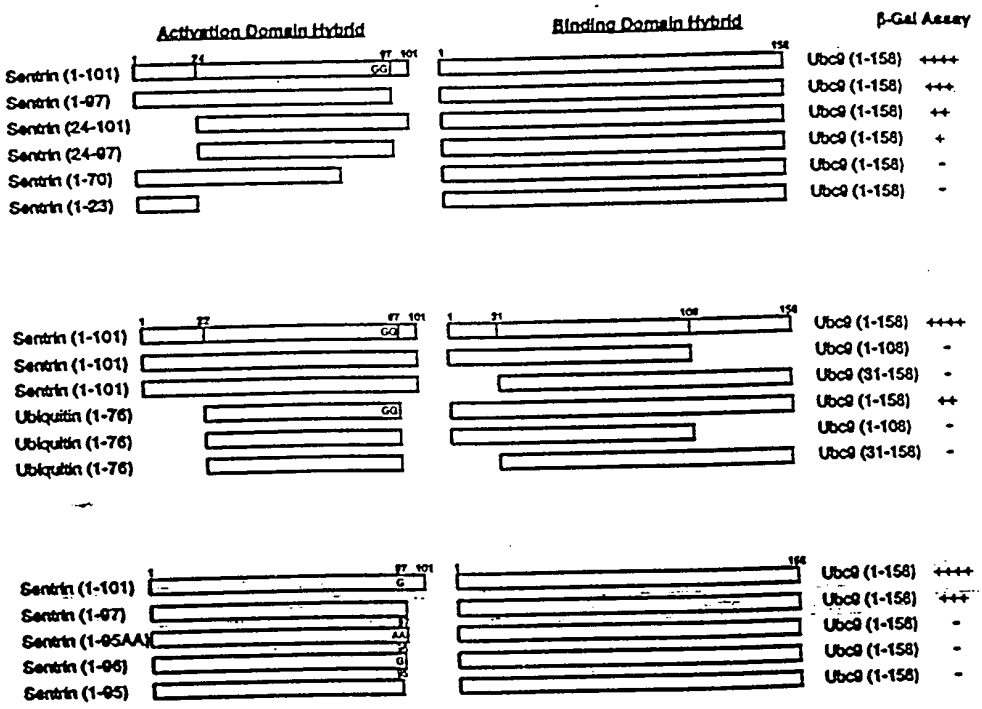
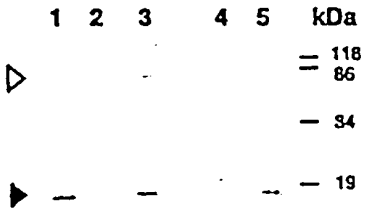


FIG. 8

FIG. 9



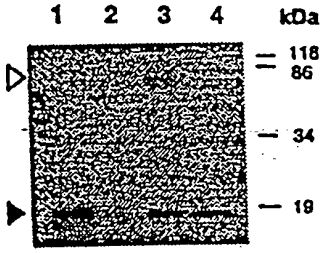


FIG. 10

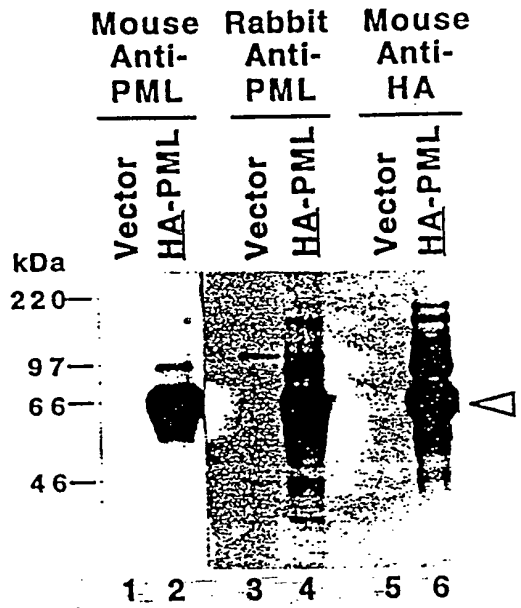

Mouse Anti-PML
Rabbit Anti-PML
Mouse Anti-HA
Vector
HA-PML
Vector
HA-PML
Vector
HA-PML
kDa
220
97
66
46
1 2 3 4 5 6

FIG. 11A

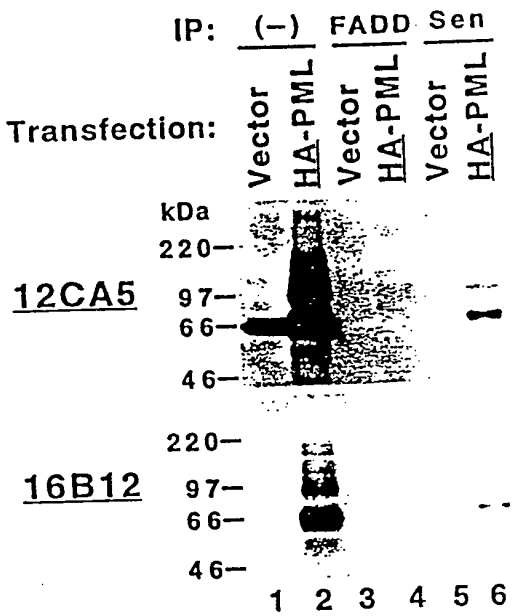

IP: (−) FADD Sen
Transfection: Vector HA-PML Vector HA-PML Vector HA-PML
kDa
220
97
66
46
12CA5
220
97
66
46
16B12
1 2 3 4 5 6

FIG. 11B

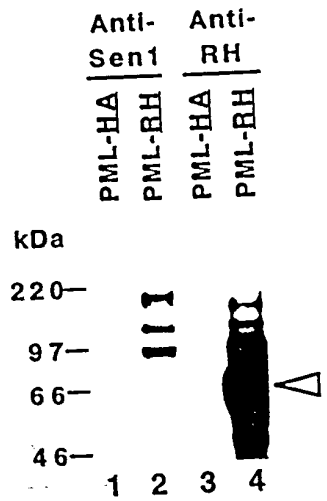

Anti-
Sen1
Anti-
RH
PML-HA
PML-RH
PML-HA
PML-RH
kDa
220
97
66
46
1
2
3
4

FIG. 11C

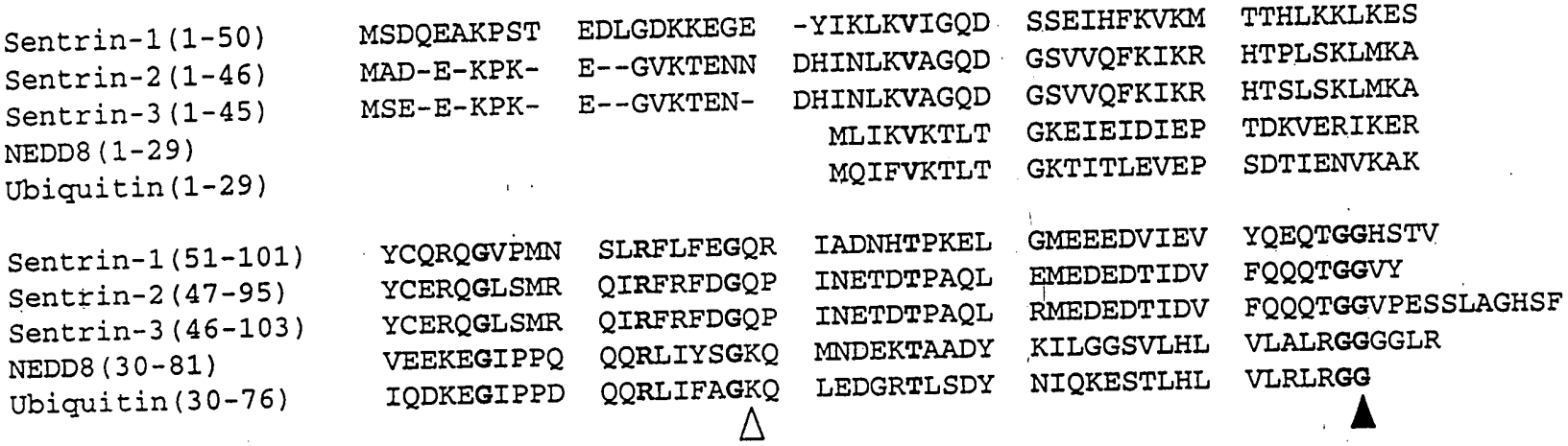


FIG. 12

09484964.011800

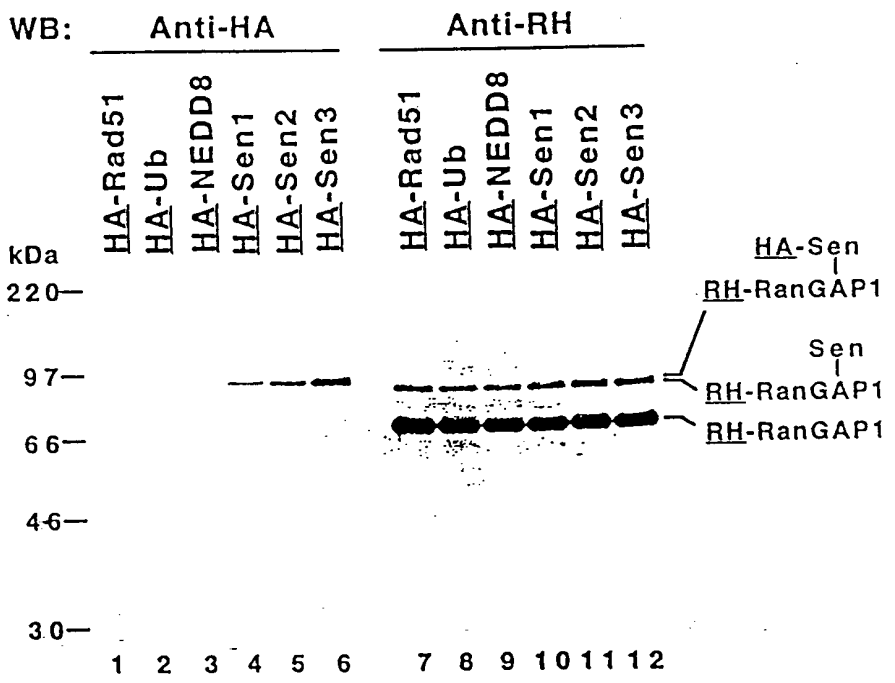

WB:
Anti-HA
Anti-RH
HA-Rad51
HA-Ub
HA-NEDD8
HA-Sen1
HA-Sen2
HA-Sen3
HA-Rad51
HA-Ub
HA-NEDD8
HA-Sen1
HA-Sen2
HA-Sen3
kDa
220
97
66
46
30
HA-Sen
RH-RanGAP1
Sen
RH-RanGAP1
RH-RanGAP1
1 2 3 4 5 6 7 8 9 10 11 12

FIG. 13A

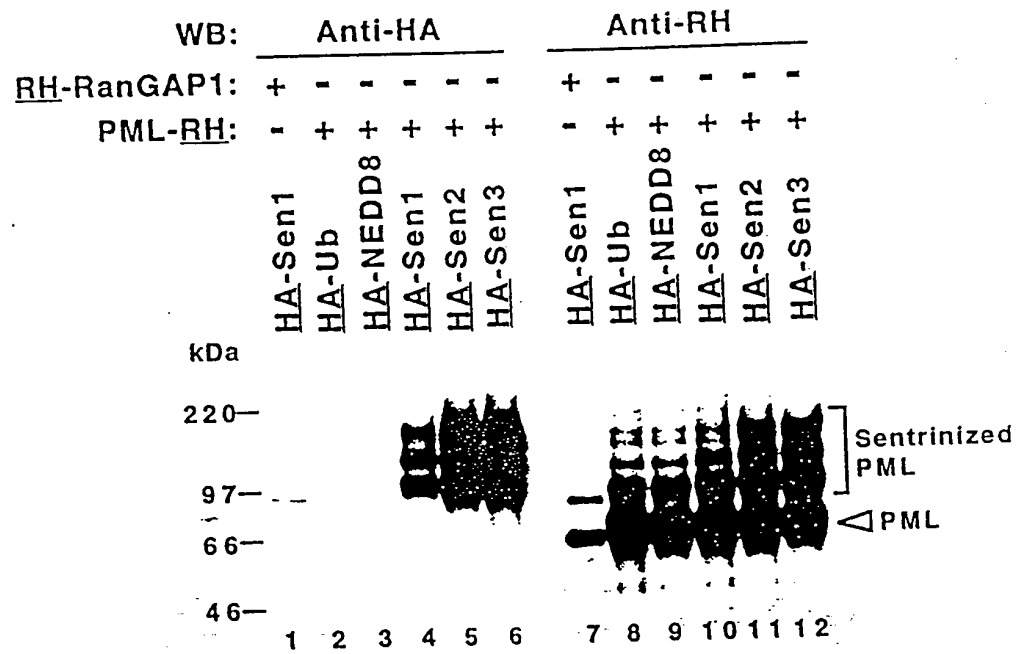

WB:
Anti-HA
Anti-RH
RH-RanGAP1:
PML-RH:
HA-Sen1
HA-Ub
HA-NEDD8
HA-Sen1
HA-Sen2
HA-Sen3
HA-Sen1
HA-Ub
HA-NEDD8
HA-Sen1
HA-Sen2
HA-Sen3
kDa
220
97
66
46
1 2 3 4 5 6 7 8 9 10 11 12
Sentrinized PML
PML

FIG. 13B

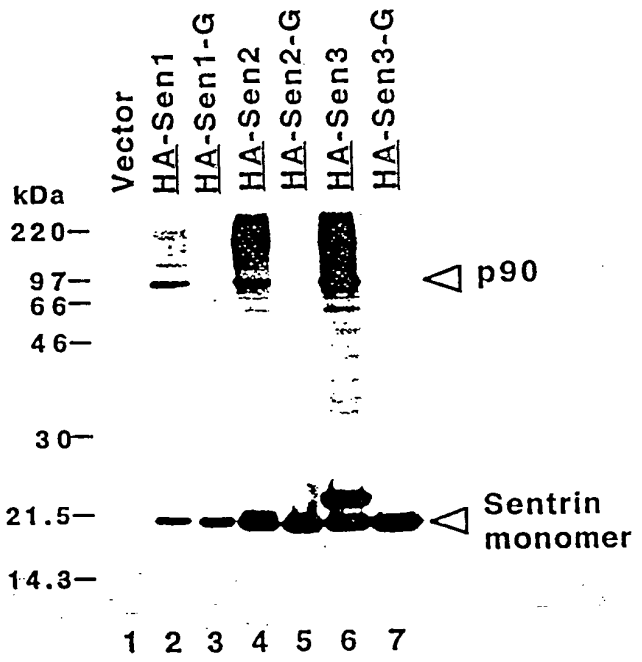

Vector
HA-Sen1
HA-Sen1-G
HA-Sen2
HA-Sen2-G
HA-Sen3
HA-Sen3-G
kDa
220
97
66
46
30
21.5
14.3
p90
Sentrin monomer
1 2 3 4 5 6 7

FIG. 13C

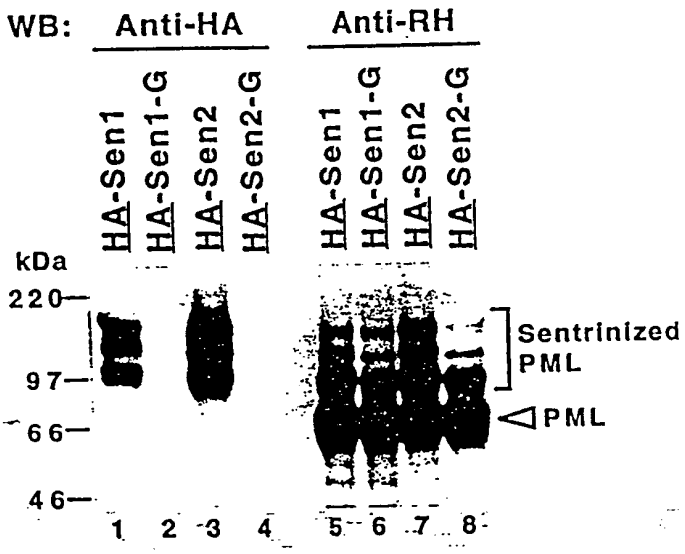

WB:
Anti-HA
Anti-RH
HA-Sen1
HA-Sen1-G
HA-Sen2
HA-Sen2-G
HA-Sen1
HA-Sen1-G
HA-Sen2
HA-Sen2-G
kDa
220
97
66
46
Sentrinized PML
PML
1 2 3 4 5 6 7 8

FIG. 13D